IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Vilmos Kéri et al.

Group Art Unit 1808
Examiner Irene Marx

#14
Dq
6/7/02

Serial No. 08/269,150

Filed on June 30, 1994

For PROCESS FOR THE ISOLATION, etc.

COPY OF PAPERS ORIGINALLY FILED

Attorney's Docket: 100-004/GPK

Hon. Commissioner of Patents and Trademarks
Washington DC 20231

DECLARATION UNDER 37 C.F.R. 1.132

My name is Dr. Kálmán Pólya. I graduated as a chemist *Summa cum Laude* in 1954. I obtained a doctorate in chemistry in 1963, and since 1979 I have been a Candidate of Sciences of the Academy of Sciences of Hungary.

I have been employed by Biogal Pharmaceutical Company in 1955 engaged primarily in fermentation and biotechnology research. As Head of the Biological Research Department I took part and supervised the developmental of process technology for mevinolin, cyclosporin and a number of other pharmacologically active substances. I have 48 scientific publications, a number of patents and have extensively lectured, and wrote 10 books.

I am presently senior scientist at Biogal Rt., with responsibility for coordination of new R&D strategies and transfer of technology from research to production.

I have been shown the text and claims of the U.S. patent application identified in the heading of this declaration (hereinafter referred to as "present application"). I have also been shown and am familiar with U.S. patent No. 5,403,728 to Jekkel et al. having a number of inventors in common with the present application. The present application is directed to a simpler and much better process than the Jekkel et al. patent, and at somewhat different pH levels.

Two samples were prepared and subjected to HPLC chromotagraphy under my supervision. At the retention times (RT) shown in the following table, the areas under

the two curves were compared and the contaminants were determined in the two samples in addition to the active ingredient.

The first sample was prepared in accordance with Jekkel et al. U.S. patent No. 5,403,728, Example 1, wherein the fermentation liquor was adjusted to pH 2, the mycelium was filtered and extracted with acetone, the acetone solution was evaporated and the residue was taken up in i-butyl acetate.

The sample of this application was prepared by adjusting the fermentation liquor to pH 9, the mycelium was filtered off and acidified to pH 2, the precipitate was filtered off and taken up in i-butyl acetate. The following results were obtained:

Retention time	Jekkel, et al.	This app.
10.18 (mevinolin)	37.92	51.842
3.05 (i-butyl-ac)	21.627	33.534
1.24	0.367	0.339
1.56	1.444	0.511
1.72	1.003	0.558
2.13	10.959	2.434 !
4.93	10.820	3.778 !
7.49	0.787	0.198
8.45	5.17	1.811 !

The foregoing table shows only those contaminants which occur in both samples. The table also shows the substantial differences, especially in the lines marked with the exclamation marks, demonstrating the low contaminant levels produced by the present invention and thus its superiority over that of Jekkel et al.

There are also additional contaminants in Jekkel et al. sample which are not present at all or at best in trace amounts present in the sample in accordance with the present application. The chromatogram of the Jekkel et al. sample showed a total of 24 peaks, but only the 9 peaks referred to in the foregoing table were in common with the present application. Thus it is conclusively established that the direct separation process of the present invention results in a surprisingly and unexpectedly pure product.

The process of the present application (Example 2), was compared with both Examples 1 and Examples 2 of the Jekkel et al. patent, and also with to another known extraction process from U.S. patent No. 4,342,767. A side-by-side comparison of the process steps shows that the process of the present application is much simpler then the known processes

US Pat. No. 4,342,767, Ex. 6	Jekkel et al., Ex. 1	Jekkel et al. Ex. 2	Pres. appln. Ex.2
fermentation liquor	fermentation liquor	fermentation liquor	fermentation liquor
filter mycelium	adjust acid pH	adjust basic pH	adjust basic pH
adj. filtered pH to 4	filter mycelium	filter mycelium	filter mycelium
extraction	extract mycelium	ion exchg. filtered liqu.	-
evaporate extract	evaporate extract	eluate with solvent mix	-
chromat. purif. (1)	diss. residue & wash.	evap. eluate rmv. solvt.	-
eluate	evaporate	adj. acid pH. of aq. phase	adj. acid pH & filter
chromat. purif. (2)	chromat.purif.eluate	extractn. evap. extrt.	
eluate	evap. eluate	diss. resid., carb.+filter	diss. resid., carb.+filter
evap. eluate	recrystallize	evaporate	evaporate
recrystallize	-	recrystallize	recrystallize

As it can be seen from the foregoing table, there is for example no need for the ion exchange purification of the filtered liquor, as it is required in the prior art, or for the extraction of the mycelium, or for the chromatographic column purification step, or for the high volume dilute fermentation liquor solvent extraction, or for the evaporation of the extract, and for the two successive chromatographic column purifications. All this makes it very clear that the process of the present application not only results in a much purer product of higher yield, but this is also accomplished in a much simpler manner than by the known processes.

I note from the foregoing findings, that one source of the advantages of the present application is likely that the mevinolin is present in the filtered fermentation liquor independently of the fact that it is partly there as a hydroxy acid and partly in its lactone form, and it can be separated with very good efficiency in an acidic pH range of 1-4.5 and then can be easily filtered off. The resulting precipitate obtained by the filtration is surprisingly clean especially when considering that it was processed in such a more simple manner then the known processes.

The extraction of mevinolin at an acidic pH is known, such as from Jekkel et al. where an aqueous solution containing mevinolin is adjusted to pH 2 and then extracted with ethyl acetate. This, however, does not suggest to a skilled chemist the process of the present application, but rather only suggests that mevinolin will not separate at an acidic pH because it is not filtered but rather extracted.

The recovery at a good yield at an acidic pH and the ability to filter it out from the filtered fermentation liquor are unexpected, because one cannot readily expect that the material would be so readily recoverable from such dilute solution, and also that it is recovered in a much cleaner form than any other product can be recovered, such as one which is obtained by extraction.

There is no fermentation processing technology known to me in which the active ingredient can be directly removed from the fermentation liquor. There have been a number of known attempts, but the end result was always that the separated product was obtained with a very low yield, or was too contaminated for further economical processing, or purification. Therefore, the process of the present invention produces a surprising conclusion in contrast to that which could be expected by persons skilled in the art, and thus brings about a new and unexpected result.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any registration issuing thereon.

April 22, 1996

Kálmán Pólya